

Study of seed proteins pattern of *brassica napus* varieties via sodium dodecyl sulfate polyacrylamid gel electrophoresis

Mehdi Kakaei¹ and Danial Kahrizi²

1- Plant Breeding Dept. (M.Sc), Faculty of Payame Noor University, Iran

2- Dept. of Agronomy and Plant Breeding Razi University. Kermanshah, Iran

Canola (*Brassica napus* L.) is an oilseed crop adapted to various environments. The current research was conducted to determine the seed storage protein profiles of 12 *Brassica napus* cultivars (Geronimo, Celecious, Milena, Sahara, Sunday, Zarfam, Dante, SLM-046, Talaye, Talent, ARC-2, Opera) that were analyzed by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) based on biochemical markers. For this purpose total soluble proteins were resolved on 15% SDS polyacrylamide gels. Then seed protein was extracted by extraction buffer. The results of SDS-PAGE were shown 17 bands on gel that they had high polymorphism. Finally genotypes were clustered into three groups by applying un-weighted pair group mean analyses.

Key Word: *Brassica napus* L., Seed, SDS-PAGE, Cluster analysis.

INTRODUCTION

The electrophoresis of seed storage protein is a method to investigate genetic variation and to classify plant varieties [Isemura et al., 2001]. The technique of Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is commonly used for separation of seed storage proteins [Ullah et al., 2010]. However, the information on the SDS-PAGE on different species of *Brassica* for genetic diversity is still limited [Rahman and Hirata, 2004]. Seed storage protein profiles have also been used to study evolutionary relation of several crop plants [Ravi et al., 2003]. The collection of these genetic resources and the assessment of genetic diversity within and between landraces should have priority for varieties improvement [Sadia et al., 2009]. Research on *Brassica* germplasm could enhance the edible oil production and nutritional benefits of these crops. [Mukhlesur and Hirata, 2004] Acrylamide gel electrophoresis in the presence of sodium dodecyl sulfate has become one of the most broadly used techniques to separate and characterize proteins [Laemmli 1970].

Since, seed storage protein analysis can be a practical

tool for identification of species, varieties and cultivars.

The technique of Sodium Dodecyl Sulphate Polyacrylamide Gel (SDS-PAGE) Electrophoresis (SDS-PAGE) is commonly used for separation of seed storage proteins [Ullah et al., 2010]. Analyses of SDS-PAGE are simple and inexpensive, which are added advantages for use in practical plant breeding [Sadia et al., 2009].

The aim of the current study was to evaluate seed protein variability in different *Brassica napus* genotypes and grouping them.

MATERIAL AND METHODS

Protein Extraction, Gel Electrophoresis and Staining

This study was carried out with 12 *Brassica napus* cultivars (Geronimo, Celecious, Milena, Sahara, Sunday, Zarfam, Dante, SLM-046, Talaye, Talent, ARC-2, Opera). These varieties are cultivated more than others in Iran. The seeds were powdered separately. Cultivars seed protein were extracted with extraction buffer (Tris-HCl, pH=8.5; NP-40, 1%; PMSF, 1mM and EDTA, 1mM), following the method described by Xi and collaborators (2006) with some modifications (Kakaei et al., 2010). SDS-PAGE method in resolving gel with 15% acryl amid and stacking gel 5% acrylamid was applied for resolving of these genotypes. At the end

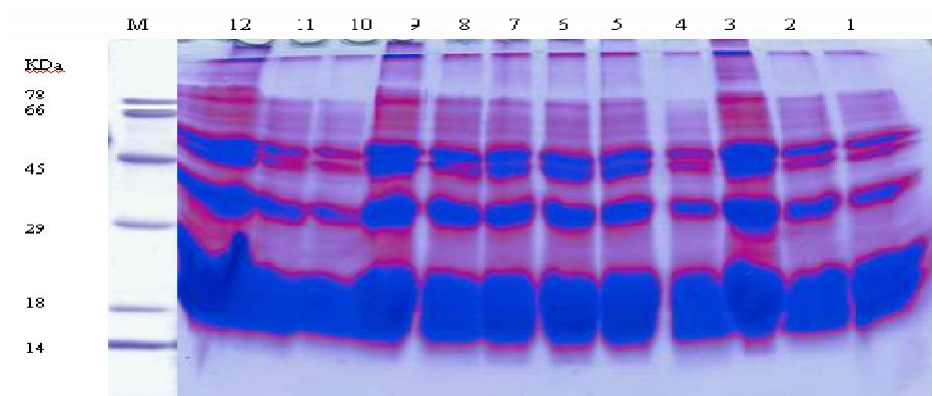


Figure 1. The SDS-PAGE result of seed proteins pattern of *Brassica napus* varieties. (1)Geronimo, (2)Celcious, (3)Milena, (4)Sahara ,(5)Sunday, (6)Zarfam, (7)Dante, (8)SLM-046, (9)Talaye, (10)Talent, (11)ARC-2, (12)Opera

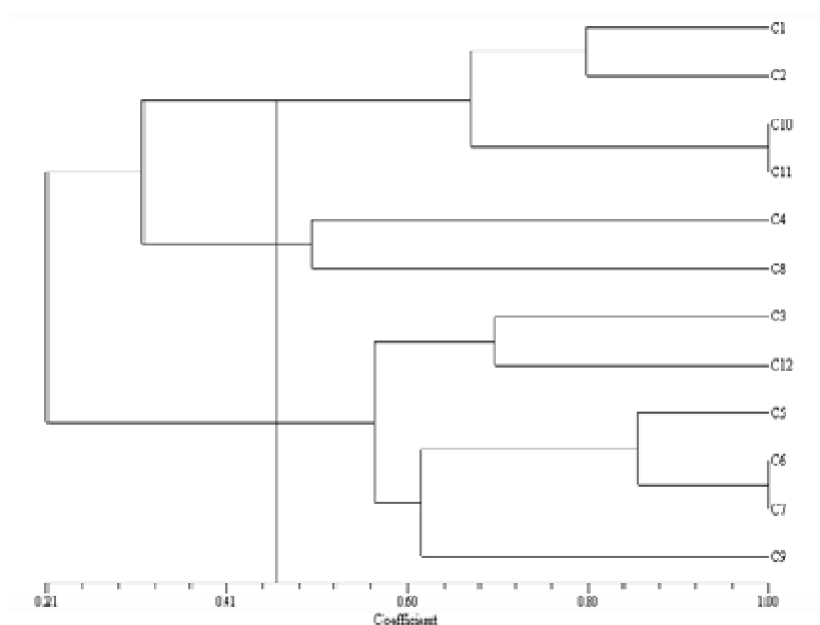


Figure 2. Dendrogram of the relationships among 12 cultivars of oilseed *Brassica napus* based on SDS-PAGE of seed storage proteins.

of electrophoresis, protein bands were revealed by Coomassie Brilliant Blue R-250 staining and destaining was done using methanol and acetic acid for 3 hours. The SDS-PAGE was adapted after Laemmli (1970) with some modifications. Protein assay was made according to Bradford (1976). The molecular weight of each band is identified by the standard proteins that their molecular weights were: Ovotransferrin (78 kDa), bovine serum Albumin (66 kDa), Ovalbumin (45kDa), Actinidin (29kDa), β Lactoglobulin (18 kDa) and Lysosyme (14 kDa).

For grouping of genotypes cluster analysis was done and dendrogram drawn based on Jacard's similarity coefficients and UPGMA (unweighed pair group mean analyses) method.

RESULTS AND DISCUSSION

Electrophoresis of proteins is a powerful tool for detection of the genetic diversity and the SDS-PAGE of seed

protein is particularly considered as a reliable technology because seed storage proteins are highly independent of environmental fluctuations [Iqbal et al., 2005 and Javid et al., 2004].

Figure 1 showed gel SDS-PAGE, *Brassica napus*. It was detected 17 bands per cultivars in electrophoregrams. The showed polymorphism is on the basis of difference in protein intensity among genotypes. The mostly of diversities such as (added or removed bands) were absorbed in areas with 66-100 kDa and 35-25 molecular weight. The bands with about 35 kDa molecular weight in genotypes numbers 1, 2, 4 and 10 were weaker than other genotypes. The polymorphism in the 15-27 kDa range in most major genotypes is not detectable. Use to SDS-PAGE is reported research [Kakaei et al., 2009: Kakaei and Farshadfar., 2010: Hameed et al., 2009:

Table 2. The *Brassica napus* genotypes that have been used in study of seed proteins pattern.

Number	genotypes name	Cluster No.
1	Geronimo	3
2	Celecius	3
3	Milena	1
4	Sahara	2
5	Sunday	1
6	Zarfam	1
7	Dante	1
8	SLM-046	2
9	Talaye	1
10	Talent	3
11	ARC-2	3
12	Opera	1

Farshadfar and Farshadfar 2008: , Kakaei 2009 and Sadia et al., 2009). The relationships among 12 cultivars of oilseed *Brassica napus* based on SDS-PAGE of seed storage proteins has been showed in figure 2 as a dendrogram. The Dendrogram divided genotypes in three clusters. Cluster 1, 2 and 3 comprises of four, two and six genotypes respectively (Table 2). This result showed that there is enough variation for seed protein content among the rapeseed cultivars and we can cross the cultivars that are the most distance (for example cross between 1 and 9) for maximum heterosis achievement.

ACKNOWLEDGEMENTS

Kermanshah University of Medical Science Medical Biology Research Center, for using laboratory protein electrophoresis.

REFERENCE

- Bradford MM (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72:248-254.
- Farshadfar M, Farshadfar E (2008). Genetic Variability among Lucerne Cultivars Based on Biochemical (SDS-PAGE) and Morphological Markers. *J. of Applied Sci.* 8(10): 1867-1874.
- Hameed A, Shah T M, Atta BM, Iqbal N, Haq MA, Ali H (2009). Comparative Seed Storage Protein Profiling Of Kabuli Chickpea Genotypes. *Pak. J. Bot.*, 41(2): 703-710.
- Iqbal SH, Ghafoor A, Ayub N, (2005). Relationship between SDS-PAGE markers and Ascochyta blight in chickpea, *Pakistan Journal of Botany*, 37(1), 87-96.
- Isemura T, Shiyo N, Shigeyuki M, Michihiro Y, Hiroo N, Masayoshi I, Osamu K (2001). Genetic variation and geographical distribution of Azuki bean (*Vigna angularis*) landraces based on the electrophoregram of seed storage proteins. *Breed. Sci.*, 51: 225-230.
- Javid A, Ghafoor A, Anwar R (2004). Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. *Pakistan Journal of Botany*, 36(1), 25-29.
- Kakaei M (2009). Effects of Genotype and drought stress on physiological, morphological, phenological and biochemical traits of winter rape (*Brassica napus* L.). M.Sc. Thesis, Islamic Azad University, Branch of Kermansha, Iran.
- Kakaei M, Mostafaie A (2010). Study of seed proteins pattern of *Cicer arietinum* by Sodium dodecyl sulfate polyacrylamid gel electrophoresis. The 3rd Iranian Pulse Crops Symposium, 19-20 may.
- Kakaei M, Farshadfar M (2010). Study of Biochemical pattern of Protein bands Chick pea Seed Varieties Grown In Controlled Environmental Conditions and Drought Stress. The National Proteomics Congress, Shiraz University, 28-29 Oct 2010. p 196.
- Kakaei M, Mostafaie A, Zebajadi A R (2009). Study of Protein Pattern of Nine Genotype of *Brassica napus* by Sodium Dodecyl Sulfate Polyacrylamid Gel Electrophoresis. Abstract book. The 3rd Iranian Proteomics Congress Pasteur Institute of Iran. 26-27 May 2010. P. 65.
- Kakaei M, Kahrizi D, Mostafaie A (2010). Study on powdery mildew disease related proteins expression in winter wheat cultivars via SDS-PAGE. *Bih. Bio. Vol. 4, No.2, Pp.:* 169-171.
- Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nat.* 227, 680-685.
- Mukhesur RMD, Hirata Y (2004). Genetic Diversity in *Brassica* Species Using SDS-PAGE Analysis. *J. of Biol.* 4(2): 234-238.
- Rahman MM, Hirata Y (2004). Genetic diversity in *Brassica* species using SDS-PAGE analysis. *J. Biol. Sci.*, 4(2): 234-238.
- Ravi M, Geethanjali S, Sameeyafarheen F, Maheswaran M (2003). Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. *Euphytica*. 133: 243-252.
- Sadia M, Malik SA, Rabbani MA, Pearce SR (2009). Electrophoretic Characterization and the Relationship between Some *Brassica* Species. *Electronic J. of Bio. Vol. 5(1):* 1-4.
- Ullah I, Ahmad Khan I, Ahmad H, Sul- Gafoor S Gul, Muhammad I, Ilyas M (2010). Seed storage protein profile of rice varieties commonly grown in Pakistan. *Asian J. of Agri. Sci.* 2(4): 120-123.
- Xi, J., Wang, X., Li, S., Zhou, X., Yue, L., Fan, J., Hao, D. (2006). Polyethylene glycol fractionation improved detection of lowabundant proteins by two-dimensional electrophoresis analysis of plant proteome. *Phytochem.* 67: 2341-2348.